

## SPECIFICATION AMENDMENTS

Please amend the specification as follows:

Page 10, please substitute the following brief description of the drawings for Figs. 4 and 5, lines 14 through 17:

Fig. 4 is a cartoon schematic rendering of a chlorosome of *C. aurantiacus* in place ~~in~~ on a cytoplasmic membrane;

Fig. 5 is a diagrammatic (cartoon) illustration of an  $RC^+$  whole cell fragment ~~chlorosome~~ of the bacterium *C. aurantiacus* and its chlorosome  $RC^-$

Page 10, please substitute the following brief description of the drawings for Figs. 8 and 9, lines 22 through 24:

Fig. 8 is a functional block diagram in the form of a flow chart of optical interactions of the components of the ~~chlorosome~~ whole cell fragment shown in Fig. 5;

Fig. 9 is a plot of absorbent spectra data for the ~~chlorosome~~ whole cell fragment of Fig. 5;

Pages 12 through 15, please substitute the following paragraphs beginning at line 20 on page 12 and continuing through line 2 on page 15:

A freeze fracture image of *C. aurantiacus* by scanning electron microscopy (SEM) was taken and is reproduced in Fig. [[1]] 3. In the image small ovals can be resolved. These are the cell's chlorosomes.

At this size scale however, reduction would require specialized EM or other imaging techniques. Thus far, no high resolution structural information has been successfully obtained on individual chlorosome ~~structures~~ structures, as such a cartoon schematic representation of the ~~chlorosome~~ whole cell fragment 100 in situ is presented in Fig. [[2]] 4. There, the whole cell fragments ~~The chlorosomes~~ 100 are depicted in place in a cytoplasmic ~~membrane~~ 95. A

proposed model of a single chlorosome unit whole cell fragment 100 is shown enlarged in Fig. [[3]] 5 in a 3-D cartoon. From the work of Blankenship, et al., the chlorosome whole cell fragment is comprised of four major sub-units: a Bchl c portion 101, a Bchl baseplate 102, a B808/866 protein 103 and a reaction center (RC) 104.

A chlorosome 110 of the *C. aurantiacus* bacterium is depicted in Fig. [[5]] 6. It includes ~~four~~ two major supra-molecular pigment-protein subunits. These are the bacteriochlorophyll (Bchl) c 101, [[a]] and the supra-molecular baseplate complex 102, ~~a B808/866 supra-molecular complex 103 and a reaction center 104~~. In its form shown in Fig. [[3]] 5 the whole cell fragment of C. aurantiacus chlorosome 100 is here designated  $RC^+$  (meaning with RC and B808/866 light harvesting apparatus). As depicted in Fig. [[4]] 6 at 110, stripped of its associated reaction center and B808/866 supra-molecular complex 103, [[it]] the chlorosome of *C. aurantiacus* is designated  $RC^-$  (meaning without RC and B808/866 light harvesting apparatus). Each sub-unit of the chlorosome whole cell fragment 100 illustrated in Fig. 5 is composed of a large number of wavelength-specific light absorbing and transducing molecules.

The first sub-unit involved in light transduction is a lipid sack 101 containing bacteriochlorophyll (Bchl) c, which is organized in units of approximately 10,000 molecules that form rod-like structures 115 (Fig. 7). As represented in the flow chart of Fig. 8 at 115, these molecules transduce photonic energy associated with 740 to 750 nm light in approximately 16 ps with very little loss. Photonic energy at 750 nm is then transduced at 117 by the baseplate membrane of the baseplate 102, which is comprised of approximately 500 molecules of Bchl a, to 795 nm to 800/810 nm in 41 - 175 ps. The B808/866 complex 103 contains 10 - 20 Bchl a molecules, which absorb at 119 at 808 and 866 nm and transfer at 883 nm in approximately 250 ps. Finally, the last stage is where, at 121, a special pair of Bchl a molecules called of the

reaction center (RC) 104, convert the light energy into chemical (photochemistry) to emit photons.

Fig. 9 plots absorbance spectra data of isolated chlorosomes RC<sup>-</sup> of *C. aurantiacus* noting peaks of interest. There, an absorbance peak at 740 - 750 nm attributable to the Bchl c rods 113 appears. A peak at 795 nm associated with the Bchl a baseplate is shown. In addition absorption of light in the blue region by the carotenoids is evident and blue secondary absorbance peaks from the Bchl c and a (designed as Soret peaks) occur. A peak attributable to the monomeric form of Bchl c (like its Soret) has a different absorbance wavelength peak than the oligomeric form that comprises the rods 113 in the chlorosomes. Like the Bchl a baseplate peak, the Bchl c oligomeric c peak is in the near infrared (NIR).

Intact *C. aurantiacus* bacteria display a unique adaptive ability to reversibly and enzymatically assemble and disassemble the foregoing structures to protect the organism from photo-induced damage. As is expected, the spectral peaks of Fig. [[5]] 6 are highly related to growth conditions of the whole cell *C. aurantiacus* bacteria. There are also related to the isolation techniques that result in purified chlorosomes. An abbreviated form of the important basic mechanisms of energy transfer that occur between the molecules of the RC<sup>-</sup> chlorosome are as depicted in Fig. 10.

The carotenoids have been shown to also transfer energy to the Bchl c oligomeric rods as is true of the Soret band (a strong absorbance of a chlorophyll in the blue region of light). However, there are subtle differences in the Bchl c found in the chlorosomes. The Bchl c found in *C. aurantiacus* chlorosomes are self-assembled (from monomeric form) into oligomeric rods. This results in a shift of the normal Soret (and  $Q_y$ ) band into a redder form. The Bchl a found in the baseplate also has a Soret region in its photonic (blue) spectra. Carotenoids can begin to

quench the structures [[ad]] and should be closely watched, as this would cause the device of this invention to operate at lower efficiencies.

Pages 15 through 16, please substitute the following paragraphs beginning at line 10 on page 15 and continuing through line 10 on page 16:

The microslide employed allowed for relatively straightforward application of the chlorosomes. This particular slide has two frosted rings on its surface, one of which is indicated at 131 in Fig. 11. The frosted ring was just sufficiently high above the surface of the slide 120 that a drop of the liquid suspension containing the chlorosomes was retained. The cover glass 118 was rested on the ring 131 and when the suspending liquid had evaporated leaving the chlorosomes adherent to the hydrophobic borosilicate cover glass surface as shown, the epoxy seal 121 was applied. The microwells slide was useful in another respect. Having two of the frosted rings 131, it permitted for the side-by-side construction as illustrated in Fig. 11 and a control. The control could be an identical silicon photovoltaic cell illuminated through the slide 120 and a further glass 118 but absent the chlorosomes, or the control could be as illustrated in Fig. 11 but having the  $RC^+$  ~~chlorosomes~~ fragments entrapped.

In the arrangement of Fig. 11, the chlorosomes  $RC^-$  and the light receiving surface of the photovoltaic cells were no more than a millimeter apart. As indicated in Fig. 11, the construction of the off-the-shelf photovoltaic cell placed the light receiving surface 133 of the silicon semiconductor in a metal housing or can 135 to be exposed through a glass closure 137.

Page 17, please substitute the following paragraph beginning at line 3 and ending on line 7:

First, the biological component (the  $RC^-$  chlorosomes), as well as controls, had to be isolated or purchased. Next, several types of characterization had to be performed (and

developed in some cases) so that the device fabrication could be accomplished. These involved many steps (and iterations) until sufficient materials were readily available (in the correct form) for use in the hybrid device configuration.

Page 17, please substitute the following paragraph beginning at line 20 and ending on page 18 at line 4:

RC chlorosome Chlorosome isolation (Gerola, 1986) starts with cells are concentrated (600 ml) by centrifugation at 3,600 xg for 60 min. 2M NaSCN with 10 mM ascorbic acid in 10 mM Pi buffer (6.5 ml monobasic: 43.5 ml dibasic phosphate buffer per liter) was added to the weighed pellet in 4ml/gm amounts. Cells were homogenized 10x in a cell disruptor/homogenizer (Fisher Scientific). Disruption of cells was performed by (one) pass in a 4°C stored French Press (ThermoSpectronic) cell with 20,000 psi. DNase I (Sigma) was added and the solution was incubated for 30 min at room temperature. The solution was passed through the cell two more times.

Page 18, please substitute the following paragraph beginning at line 12 and ending at line 15:

After isolation of the RC chlorosomes from *C. aurantiacus* whole cells, the chlorosomes were (at various dilutions into Tris buffer) tested as in the above methods. This was performed in order to assess quality control by comparing spectral data (on absorbance) and relative output (emission).

Page 19, please substitute the following paragraph beginning at line 1 and ending at line 10:

Another technique utilized the evaporation procedure as well as an aqueous method to allow incorporation of the RC<sup>+</sup> chlorosomes onto a glass surface. Both techniques start with taking 0.5  $\mu$ l of a known concentration of the chlorosomes and placing it onto a borosilicate glass coverslip (Fisher Scientific). In the evaporation method, evaporation, under vacuum, is performed overnight and then the sample is sealed onto a fluorescent antibody microslide (Fisher Scientific). In the physical adsorption method, the slide is prepared in the aqueous phase and inverted during sealing, thus allowing for ensuring a hydrated sample as well as diffusion of the chlorosomes onto the surface of the hydrophobic glass. Samples were also studied under laser scanning confocal microscopy (LEICA) to investigate orientation and function (stability) was observed with absorbance spectroscopy of the sample afterwards.

Page 21, please substitute the following paragraph beginning at line 5 and ending at line 18:

Another imaging technique, namely Atomic Force Microscopy (AFM) was performed by evaporating a 100  $\mu$ l sample of chlorosomes (overnight in desiccant jar) onto a standard borosilicate coverglass. A Digital Instruments' Nanoscope III Multimode AFM was used in Tapping Mode (TMAFM) to image the chlorosomes at various dilutions. Again, the dilutions' absorbance spectra were taken prior to imaging. Prior to running the AFM experiments, a known liquid volume (400  $\mu$ l) was taken from solution containing RC<sup>+</sup> chlorosomes (~~no reaction center~~) in DI water previously characterized via absorbance spectra (ABS = 0.01 @740 nm) and was evaporated onto a clean, optically clear glass disk with known surface area (113.1mm<sup>2</sup>). The disks were made hydrophobic to enhance RC<sup>+</sup> attachment and orientation due to theoretical studies performed by using a molecular modeling algorithm (Chou, 1977) that suggested that the baseplate region attached to the reaction center may be hydrophobic in nature. Tapping mode

AFM experiments were conducted utilizing a small scan head (D head) to scan  $1\mu\text{m}^2$  surface areas on both the control disks (no  $\text{RC}^-$  chlorosomes deposited) and test disks ( $\text{RC}^-$  chlorosomes deposited).

Page 30, please substitute the following paragraph beginning at line 12 and ending at line 18:

Processing of the chlorosome requires isolation of the chlorosomes from the whole cell walls as described above and as. This is done using a procedure well documented in the literature. ~~although certain factors do arise in the process. There are different procedures used to isolate chlorosomes without the reaction centers ( $\text{RC}^-$ ) versus those with ( $\text{RC}^+$ )~~. The solvents, agents, and buffer types used in the procedure are also very important and factors such as (the type, molarity, ionic strength, pH, and strength) all come into play. These factors will affect the state of aggregation and purity (and success) of the isolated chlorosomes.

Page 31, please substitute the following paragraph beginning at line 3 and ending at line 6:

Again, product final assembly is addressed in the first section above. However, other issues pertaining to lifetime of device and/or other issues such as post fabrication storage include factors of temperature, light intensity (and quality – i.e. wavelength) and # days, to name but a few.

Page 41, please delete the following paragraph beginning at line 14 and ending at line 21:

~~Processing of the chlorosome requires isolation of the chlorosomes from the whole cells. As indicated above, this is done using procedures well documented. Nevertheless certain factors need to be taken into account during this process. These are the different procedures used to~~

isolate chlorosomes without the reaction centers (i.e. the RC<sup>-</sup> chlorosomes vs. the RC<sup>+</sup> chlorosomes). Solvents, agents and buffer types used in the procedure are also important, and factors such as the type, molarity, ionic strength, PH and strength of these all come into play. These factors will affect the state of aggregation impurity of the isolated chlorosomes, and consequently the ultimate success of the design.